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Mutations in *LOXHD1* gene can cause auditory neuropathy spectrum disorder

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Abstract

Objectives: The aim of this paper was to study the auditory phenotype of three related children with sensorineural hearing loss (2 sisters and their cousin) following genetic analysis revealing mutations in *LOXHD1*.

Methods: Genetic testing was conducted on three related children. They were assessed with a standard clinical test battery including distortion otoacoustic emissions, auditory brainstem responses and audiometry.

Results: We identified heterozygous variants in *LOXHD1* in a family of Irish/German and Italian/Irish ancestry with autosomal recessive auditory neuropathy spectrum disorder (ANSO). Mutations in *LOXHD1* (MIM #613072) have been linked to an autosomal recessive nonsyndromic hearing loss (DFNB77), mapped to the locus 18q12-q21. All three subjects had evidence of some, albeit few, functioning cochlear hair cells as revealed by the presence of a cochlear microphonic and/or partial otoacoustic emissions early in life.

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Ethical statement

The work described in the manuscript "Mutations in *LOXHD1* gene can cause Auditory Neuropathy Spectrum Disorder" has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and written informed consent was obtained for all subjects (Nemours Institutional Review Board).

Conclusion: To our knowledge, this is the first association between *LOXHD1* mutations and ANSD in two patients who have been successfully managed with cochlear implants.

Keywords

Auditory neuropathy; *LOXHD1*; Cochlear microphonic; Cochlear implants

1. Introduction

Auditory neuropathy spectrum disorder (ANSD) represents a family of hearing disorders with a continuum of behavioral presentations, ranging from mild difficulty processing speech to profound hearing loss. ANSD, first described by Starr et al. [1], differs from other types of sensorineural hearing loss by its site of impairment that includes a dysfunction at the level of the cochlear inner hair cells, the synaptic region between the inner hair cells and the auditory nerve, or at the level of the auditory nerve itself, while the function of the outer hair cells is preserved. ANSD accounts for approximately 10%–15% of cases of permanent childhood sensorineural hearing loss [2–4]. A number of different etiologies have been associated with ANSD, including genetic conditions with syndromic or nonsyndromic patterns and autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance configurations [5].

Among known ANSD causing nonsyndromic causing mutations are those in the Otoferlin (*OTOF*) gene affecting the exocytosis of synaptic vesicles at the inner hair cell ribbon synapses (congenital nonsyndromic type of ANSD) [6,7], in the *DNFB59* gene coding for pejvakain, *AUNAI1* (*DIAPH3* gene) and *AUXN1* [8–10]. ANSD can also be associated with hereditary syndromes such as Charcot-Marie-Tooth, Leber's hereditary optic neuropathy, autosomal recessive optic atrophy, Friedreich Ataxia and Mohr-Tranebjaerg syndrome [11 for a review,12]. Although the pace of identifying genetic mutation causing hearing loss is continuously increasing, the heterogeneity of ANSD remains a challenge in understanding our genetic knowledge of the disorder. Furthermore, the phenotyping of hearing loss might not be comprehensive enough to define ANSD versus a more typical type of sensorineural hearing loss.

Here, we report the auditory phenotype of three related children with sensorineural hearing loss (2 sisters and their cousin) diagnosed with mutations in *LOXHD1*. The first association between a nonsense *LOXHD1* mutation and hearing loss was made in affected members of a five-generation consanguineous Iranian family segregating a progressive form of autosomal-recessive nonsyndromic hearing loss (DFNB77) [13]. Since then, many pathogenic variants have been reported [14]. The protein encoded by *LOXHD1* plays an important role in maintaining normal hair cell function in the cochlea [13]. Subsequent reports indicate large variations in the phenotype associated with more than 60 variants in *LOXHD1* with differences in the onset, type and severity [15–19]. These previous studies all reported mutations in *LOXHD1* as causing an autosomal-recessive nonsyndromic type of sensorineural hearing loss (SNHL). Here we report the first association between *LOXHD1* mutations and auditory neuropathy spectrum disorder (ANSD) and the successful management of two of these patients with cochlear implants.

2. Material and methods

2.1. Patients

Three related children (two sisters and their male first cousin) diagnosed with autosomal recessive hearing loss at age 2 weeks (proband), 2 months (proband sister) and 4 weeks (proband cousin) were recruited following written informed consent (Nemours Institutional Review Board) to participate in a study aiming at delineating the genetic etiology of their hearing loss.

2.2. Audiological assessment

The children were assessed with a standard clinical test battery composed of otoscopy examination, tympanometry, middle ear muscle reflexes (MEMR), distortion product otoacoustic emissions (DPOAEs; Bio-Logic AuDX Pro), auditory brainstem responses (ABRs), and behavioral tests (pure tone audiometry, speech awareness and recognition thresholds and speech discrimination scores). Several of these tests were repeated over time to monitor the progression of hearing loss. The ABRs were performed during natural sleep and/or under sedation using a 4-electrode 2-channel montage with insert earphones to present air conduction clicks (Vivosonic Integrity, Toronto, ON, Canada). The initial rate of stimulation was 27.5/sec or 37.7 and reduced to 7.6/sec in some occasions.

2.3. Genetic testing and analysis

Exome sequencing for the proband and her sister was provided by the University of Washington Center for Mendelian Genomics (UW-CMG).

2.3.1. DNA extraction—DNA extractions were performed by the Nemours Center for Pediatric Research Biomolecular Core Laboratory using Standard Operating Procedures (SCR_018265). Genomic DNA was extracted from blood using the Puregene Blood Kit (Qiagen). To confirm the genetic relationship between the proband (37–1) and affected sibling (37–2) to their parents, mother (37–3) and father (37–4), we used the AmpFLSTR Identifiler PCR Amplification Kit (ThermoFisher), containing 15 short tandem repeat loci plus Amelogenin.

2.3.2. Library production, exome capture, sequencing—Library construction and exome capture have been automated (Perkin-Elmer Janus II) in 96-well plate format. 1 µg of genomic DNA was subjected to a series of shotgun library construction steps, including fragmentation through acoustic sonication (Covaris), end-polishing and A-tailing, ligation of sequencing adaptors and PCR amplification with dual 8 bp barcodes for multiplexing. Libraries underwent exome capture using the Roche/Nimblegen SeqCap EZ v2.0 (~36.5 MB target). Prior to sequencing, the library concentration was determined by fluorometric assay and molecular weight distributions verified on the Agilent Bioanalyzer (consistently 150 ± 15 bp).

2.3.3. Clustering/sequencing—To facilitate optimal loading, all samples were sequenced on the Illumina MiSeq platform prior to deep sequencing. Barcoded exome libraries were hand pooled prior to clustering (Illumina cBot) and loading. Massively

parallel sequencing-by-synthesis with fluorescently labeled, reversibly terminating nucleotides was carried out on the HiSeq sequencer. Current throughput was sufficient to complete 16–20 multiplexed exomes per lane at high coverage (40–60X mean coverage).

2.3.4. Read processing—The sequencing pipeline was a combined suite of Illumina software and other “industry standard” software packages (i.e., Genome Analysis ToolKit [GATK], Picard, BWA, SAMTools, and in-house custom scripts) and consists of base calling, alignment, local realignment, duplicate removal, quality recalibration, data merging, variant detection, genotyping and annotation.

2.3.5. Variant detection—Variant detection and genotyping were performed using the HaplotypeCaller (HC) tool from GATK (3.2). Variant data for each sample were formatted (variant call format [VCF]) as “raw” calls that contain individual genotype data for one or multiple samples and flagged using the filtration walker (GATK) to mark sites that were of lower quality/ false positives [e.g., low quality scores (Q50), allelic imbalance (ABHet 0.75), long homopolymer runs (HRun > 3) and/or low quality by depth (QD < 5)].

2.3.6. Data analysis QC—Data QC included an assessment of: (1) total reads (minimum of 50 million PE50 reads); (2) library complexity (3) capture efficiency (4) coverage distribution: 90% at 8X required for completion; (5) capture uniformity; (6) raw error rates; (7) Transition/Transversion ratio (Ti/ Tv); (8) distribution of known and novel variants relative to dbSNP; typically, < 7% (9) fingerprint concordance >99%; (10) sample homozygosity and heterozygosity and (11) sample contamination validation. Exome completion was defined as having >90% of the exome target at > 8X coverage and >80% of the exome target at > 20X coverage. Typically, this required mean coverage of the target at 50–60X.

2.3.7. Variant annotation—The SeattleSeq Annotation Server (<http://gvs.gs.washington.edu/SeattleSeqAnnotation/>) was used as an automated pipeline for annotation of variants derived from exome data. This publicly accessible server returns annotations including dbSNP rsID (or whether the coding variant is novel), gene names and accession numbers, predicted functional effect (e.g., splice-site, nonsynonymous, missense, etc.), protein positions and amino-acid changes, PolyPhen predictions, conservation scores (e.g., PhastCons, GERP), ancestral allele, dbSNP allele frequencies and known clinical associations. The annotation process has also been automated into our analysis pipeline to produce a standardized, formatted output (VCF-variant call format).

2.3.8. LOXHD1 PCR sequencing—*LOXHD1* variants confirmations were performed on the proband, sister and their parents by the Nemours Biomolecular Core Laboratory. The regions of interest that were identified via whole exome sequencing were amplified by PCR using parameters listed below and subjected to Sanger Sequencing. PCR Primers were designed for exon 19 (p. Arg899Pro) using the following primer pair: 5'-TGCCCTTTGAACAGCTTGAGG-3' and 5'-ACGACCCACTTACGCTCAGGA-3' that amplified a 490 base pair (bp) fragment. The primer pair for exon 29 (p. Arg1494Ter) amplified a 455 bp fragment using the following primers: 5'-ACAAATCCAGTTCCTCAAGACCTAA-3' and 5'-CTCCACCTCATCCCCTATGCAG-3'.

Both primer pairs were amplified for 35 cycles at an annealing temperature of 61 °C. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher).

The cousin's proband was tested at an outside facility using a panel of 130 genes (GeneDx; Gaithersburg, MD, USA). Using genomic DNA from the subject, the coding regions and splice junctions of the genes on this test were enriched using a proprietary targeted capture system developed by GeneDx and sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina sequencing system paired-end reads. Bi-directional sequence was assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants. Capillary sequencing was used to fill in target regions not covered with sufficient depth or quality in 22 genes with high clinical sensitivity. For STRC, only deletion/duplication testing was performed. Sequencing analysis was limited to exons 1–21 of OTOA and exons 9–10 of PTPRQ. The mitochondrial genome was amplified by long-range PCR and sequenced for 6 pathogenic mitochondrial variants: MT-RNR1: m.1555 A > G; m1494 C > T; MT-TL1: m.3243 A > G; m.32911 T > c; m.7445 A > G; m.7511T > C. Two common GJB6 deletions, – 309 kb del (GJB6-D13S1830) and – 232 kb del (GJB6-D13S1854), were assessed by multiplex junction-specific PCR with primers designed to flank the breakpoints of these deletions. In addition, multiplex ligation-dependent probe amplification (MLPA) was used to detect common large deletions and duplications involving the STRC and OTOA genes. Sequence and copy number variants were confirmed by an alternate method when possible, and were reported according to the Human Genome Variation Society (HGVS) nomenclature or the International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively.

3. Results

3.1. Auditory findings

3.1.1. Proband—The proband, a female born full term with unremarkable medical and otologic histories, failed her newborn hearing screening bilaterally. Information as to whether OAEs or automated ABR were used was unavailable. Following the failed newborn screening, an ABR performed at 2 months of age revealed a diagnosis of ANSD (i.e., presence of a bilateral cochlear microphonic in the absence of neural synchrony). A repeat ABR at 12 months of age produced the same result (Fig. 1A).

Her DPOAEs were essentially absent, except for a response at 2 kHz recorded twice around 2 months of age. Behavioral observation audiometry at 6 months revealed a severe to profound hearing loss for 500–4000 Hz in the sound field condition. Testing using live speech, narrowband noise and warble tone stimuli was performed as well using insert earphones and resulted in no observable responses. Similar audiometric results in the sound field were observed 2 months later at 8 months of age. At that age, a vestibular evoked myogenic potential (VEMP; Interacoustics) testing revealed normal and symmetrical responses.

She was bilaterally fitted with loaner hearing aids at 9 months. She quickly displayed signs of discomfort with louder sounds and the overall gain and output limit were subsequently decreased for both hearing aids. A month later, responses with hearing aids to narrowband stimuli (500–1500Hz) were observed in the moderate to severe hearing loss range, while responses at 2000–4000 Hz could not be elicited.

3.1.2. Proband sibling—Born full term following an uncomplicated pregnancy, the proband sibling also failed her newborn hearing screening. The test performed was unavailable for review. She had no risk factors for hearing loss, except for her sister's history of permanent hearing loss. She was diagnosed with ANSD by ABR testing at 2 weeks of age. She had partially present DPOAEs (present at 4 and 5 kHz in the right ear at 5 kHz only in the left ear). The ABR showed a present cochlear microphonic bilaterally in response to both click polarities, with no neural responses on the right side, at the highest intensity tested (99 dBnHL) at rates of 7.6 and 37.7 per seconds. On the left side, a delayed wave V (around 12 ms) was observed at 99 dBnHL for both rate of stimulation (Fig. 1B). A potential wave III was observed around 7 ms, but only for the lowest rate of stimulation. Tone bursts testing at 0.5, 1, 2 and 4 kHz revealed that wave V was only present at high level of stimulation, i.e., between 85 and 95 dBnHL. In view of the sister's presence of hearing loss, a second ABR was not performed.

Behavioral testing at 3 and 7 months of age indicated hearing thresholds in the profound range. Hearing aids were provided to her at 7 weeks of age. She quickly displayed the same signs of discomfort as her sister with the hearing aids. She became fussy in noisy and loud environment when wearing them but would calm down once these were removed. At 7 months of age, aided testing revealed responses in the moderate to moderately-severe range for speech and narrowband noise for 250–500 Hz only as there was no aided responses from 1 to 4 kHz. A speech sound awareness (SAT) of 75 dB was obtained in the sound field.

A speech language evaluation was completed at 13 weeks of age, using the receptive and expressive emergent language test-3 (REEL-3) based on a checklist that uses observational information reported by parents. The assessment revealed significant delayed receptive and expressive language skills.

3.1.3. Cousin proband—The cousin proband also failed his newborn screening (the type of testing was similarly unavailable for review). An undated ABR was performed at 4 weeks of age. The ABR revealed the presence of waves I, III and V bilaterally with delayed latencies. A latency-intensity function was observed bilaterally. Click thresholds were 50 dB nHL in the right ear and 60 dB nHL in the left ear. Several other ABRs were performed over the next 3 years, revealing a progression of the hearing loss. At 11 months of age, ABR tone burst thresholds increased significantly (a click ABR was not obtained at that time). At 2 years of age, a click ABR showed only a wave V remaining bilaterally, with a significantly delayed latency. Thresholds decreased from 50 to 90 db eHL from 500 Hz to 4 kHz in the right ear and from 50 to 85 db eHL from 500 Hz to 4 kHz in the left ear. Between age 2 and 3, the ABR morphology and thresholds remained stable (Fig. 1C) (i.e., down sloping from 40 to 85 db eHL bilaterally). DPOAEs were present from 2 to 6 kHz in the left ear at 1 month of age, and only at 6 kHz in the right ear in presence of negative middle ear

pressure. Two repeat tests at 3 and 10 months of age indicated that the DPOAEs were absent bilaterally. At 21 months, MEMR testing revealed the presence of the reflexes bilaterally with elevated thresholds for most frequencies (equal to or above 95 dB). The most recent ear specific testing (3 years ½) revealed a slopping moderate to severe hearing loss with a SAT of 45 and 55 dB in the right and left ears respectively.

3.2. Genetic findings

The proband and her affected sister both have predicted damaging compound heterozygous variants in *LOXHD1* (Fig. 2): missense c.2696G > C (p.Arg899Pro) and stop gain c.4480C > T (p.Arg1494Ter). Their mother was heterozygous for R899P and their father for A1494T. The cousin was heterozygous for c.2696G > C (p.Arg899Pro) and c.3325A > T (p.Ile1109Phe). The mother of the cousin was heterozygous for I1109F while the father was heterozygous for R899P.

3.3. Management of hearing loss

The proband received bilateral implants at 16 months and was activated about 3 weeks later. An MRI performed prior to cochlea implantation revealed unremarkable inner ear structures, including the semicircular canals, vestibule, and cochlea. A speech and language evaluation revealed age appropriate receptive and language skills (Preschool Language Scale – 5) and age-appropriate articulation skills (Goldman Fristoe – 2 Test of articulation) at 3 years of age (2 years postimplantation). The most recent audiologic testing at 9 years of age (8 years post-cochlear implantation) indicated normal pure tone audiogram, speech reception thresholds (SRT) of 15 dB HL and scores of 100% to the CID W-22 for each ear separately as well as in the bilateral condition. She obtained a score of 100% in the right ear and of 98% in the left ear when tested in noise with a +5 signal-to-noise ratio.

The proband sibling received bilateral cochlear implants at 9 months of age. Comparably to her sister, an MRI revealed that the inner ear structures, including the semicircular canals, vestibule, and cochlea were unremarkable. Her first behavioral response (SAT) was obtained one-month post-cochlear implantation at 25 dB HL. A speech language evaluation at 2 years (16 months after CIs) demonstrated age appropriate receptive and expressive language skills. At 31 months postimplantation, thresholds between 25- and 50-dB HL were obtained between 500 and 6kHz using conditioned play audiometry due to her age and the SRT was at 10 dB HL for each ear separately. At 46 months of age (37 months post implantation), thresholds were obtained in the normal to mild range at 0.25–6 kHz and SRT at 15 dB HL. She continues to develop speech and language beautifully and is on track for her age.

The cousin proband was fitted with hearing aids at 6 months of age. His aided pure tone thresholds at 3 years ½ were in the mild range with an aided SRT of 25 dB. Although still delayed, he is currently making progress in terms of speech and language development and remains closely followed with a cochlear implant candidacy evaluation pending on the rate of language development and potential worsening of his hearing loss.

4. Discussion

We identified heterozygous variants in *LOXHD1* in a family of Irish/ German and Italian/ Irish ancestry with autosomal recessive ANSD. *LOXHD1* (MIM #613072) is a gene that has been linked to an autosomal recessive nonsyndromic hearing loss (DFNB77), mapped to the locus 18q12-q21. The *LOXHD1* protein consist of 15 PLAT (polycystin/lip-oxygenase/ α -toxin) domains, believe to be involved in targeting of proteins to the plasma membrane [20,21]. *LOXHD1* is localized in outer and inner hair cells along the plasma membrane of stereocilia and plays a crucial role in maintaining normal function of these cells. In the *samba* mice containing a missense mutation in *LOXHD1* gene [13], hearing impairment is present at 3 weeks post-birth and they become deaf at 8 weeks of age. Although the development of the stereocilia are not affected in *samba* mice, the hair cells show functional defects and they eventually degenerate resulting in a progressive type of hearing loss. In the young mice, both IHC and OHC appear to develop normally, including at the stereocilia level at the medial and apical cochlear level. At the basal turn however, some hair cells showed morphological changes, with fused stereocilia and membrane ruffling at the apical cell surface. Significant degenerative changes were noticed later on, including hair cell and spiral ganglion neuron loss. Degeneration of spiral ganglion neurons is thought to be a secondary consequence of hair cell damage as *LOXHD1* is specifically expressed in hair cells. Mutations of *LOXHD1* have been reported albeit they appear to be rare. The variants in *LOXHD1* already identified produced different types of hearing loss, varying from stable to progressive and from mild to profound. In the first report of *LOXHD1* mutation, Grillet et al. [13] identified a homozygous stop mutation, c.2008C > T (p.R670X) in exon 15 in all affected members in a five-generation consanguineous Iranian family. The affected members presented with an autosomal recessive nonsyndromic hearing loss, with preserved low frequencies and mild-to-moderate mid-and high-frequency hearing loss during childhood and adolescence, with an onset around 7–8 years of age. Here we observed a different phenotype with congenital profound hearing loss in the proband and affected sibling, while the proband cousin presented with a moderate-to-severe hearing loss from birth. All three subjects had evidence of some, albeit few, functioning cochlear hair cells as revealed by the presence of a cochlear microphonic and/or partial DPOAEs early in life. The cousin proband was the only one of the three with clear functioning OHCs at birth although his DPOAEs disappeared quickly as shown by 2 successive tests at 2 and 10 months of age. Edvardson et al. [15] reported a similar pattern to the proband and her sister, in nine patients from two unrelated, nonconsanguineous, Ashkenazi Jewish families, a single homozygous mutation in exon 30, c.4714C > T, resulting in the substitution of arginine (CGA) at codon 1572 by a premature stop codon (TGA) (R1572X). In these patients, the hearing loss was congenital and non-progressive, severe-to-profound with milder loss at frequencies below 500 Hz. ABRs were tested in 5 children in the first family and were found to be absent but there is no specification regarding the potential presence of a cochlear microphonic, therefore ANSD could not be ruled out in their study. The four other children from their second family had absent otoacoustic emissions at birth. All patients benefited from cochlear implants between 7 and 10 years of age. In Hu et al. (2018), 2 other variants (c.1751C > T [p.T584 M] and c.5815G > A [p.D1939 N]) were identified as possibly pathogenic *LOXHD1* mutations, associated with progressive nonsyndromic hearing loss. Again, there is no information

regarding the diagnostic of ANSD in their study. Shen et al. [19] found a non-progressive hearing loss in a Chinese woman (c.5948C > T) in a consanguineous family. In Minami et al. (2016), c.5674G > T (p.V1892F) and c.4212+1G > A were revealed as novel pathologic *LOXHD1* mutations causing a progressive hearing loss, worst for the mid-high frequencies than the low frequencies. None of these studies specified if ANSD was ruled out, and it is likely, based on the testing used that the search for ANSD was not carried on by using both click polarities to elicit the ABRs or by the constant use of otoacoustic emissions. Wesdorp et al. [18] studied affected individuals from 9 families and found that mainly the high frequencies were affected, although in some cases the mid frequencies and/or low frequencies are equally affected. Inter- and intra-familial variation in severity (from mild to profound) and progression of the hearing loss (stable or progressive) were observed. They could not establish a clear correlation between the type or location of the variant and the severity or progression of the hearing loss. Mori et al. [22] reported the variant c.4480C > T in a Japanese family with moderate to severe hearing loss, non-progressive. Other studies with the variant c.4480C > T have noticed a severe to profound, progressive hearing loss [see 23] and this variant is therefore considered pathogenic or likely pathogenic. Similarly, the variant c.2696G > C has been published previously in the compound heterozygote state in association with mild or moderate hearing loss [18,24]. The I1109F variant has not been published as a pathogenic variant to our knowledge.

Because DFNB77 is rare, genotype-phenotype correlation has not been well characterized and, to our knowledge, none of the published DFNB77 studies have measured the function of the outer hair cells (by means of otoacoustic emissions and/or cochlear microphonic recordings) and therefore did not distinguish ANSD as a type of sensorineural hearing loss. Here, we report 2 cases of presynaptic ANSD in the proband and her sister, based on their ABR results and the presence of outer hair cell function, albeit over a very limited frequency range and amount of time after birth. The proband cousin also had evidence of some outer hair cell function right after birth that likewise disappeared rapidly. The remaining presence of wave V in this individual, at 3 years of age, indicates that in contradiction to his cousins, a significant greater number of inner hair cells are still functioning, despite a clear loss over time, as shown by the disappearance of waves I and III.

Grillet et al. [13] observed in his study some spiral ganglion neuron loss, thought to be a secondary consequence of hair cell damage. Here, the proband and her sister have been very successful cochlear implant users which indicates that most, if not all, their spiral ganglion cells are still functioning. Since *LOXHD1* mutations affect the hair cells, this outcome is therefore not surprising for a presynaptic type of hearing loss. Edvardson et al. [15] presented 2 patients with severe-profound bilateral sensorineural hearing loss due to *LOXHD1* mutations who also benefited from cochlear implants. Similarly, in the cousin proband although the outer hair cells seem to have stopped functioning early on (2–3 months after birth), there was still enough functioning inner hair cells to activate part of the auditory nerve and central pathways as observed objectively by the presence of, albeit altered, an ABR response and MEMR. However, the synchrony was impaired enough to not elicit waves I and III, and speech comprehension appeared to be abnormal despite close to normal pure tone thresholds with amplification.

Clinical reports of *LOXHD1* mutations have not revealed any vestibular symptoms in affected individuals. Grillet et al. [13] revealed that vestibular hair cells only expressed reduced amounts of *LOXHD1* and are not affected by the mutation in the *samba* mice, which corroborate the lack of vestibular and balance issues in the developing siblings and presence of normal *VEMP* in the proband and previous observations by Wesdorp et al. [18] in 7 affected individuals.

Some variants of *LOXHD1* have been associated with late-onset Fuchs corneal dystrophy [25]. However, several studies demonstrated conflicting conclusions in that there was no association between *LOXHD1* mutations and late-onset Fuchs corneal dystrophy [18,25]. Although the relationship between *LOXHD1* and Fuchs corneal dystrophy remains unclear, ophthalmologic examinations should probably be performed in those patients with hearing loss suspected to be due to *LOXHD1* mutations.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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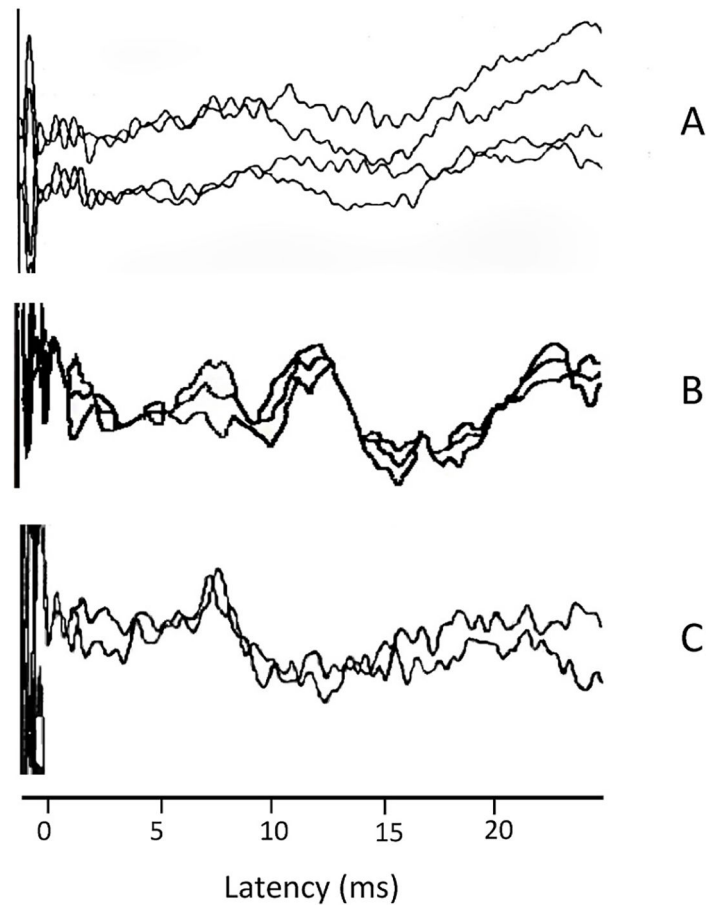


Fig. 1. Auditory brainstem responses obtained in the 3 patients following click stimulation with both rarefaction and condensation polarities: A) right ear of the proband at 12-month of age B) left ear of the proband sister at 3 weeks of age C) right ear of the proband cousin at 2 years of age.

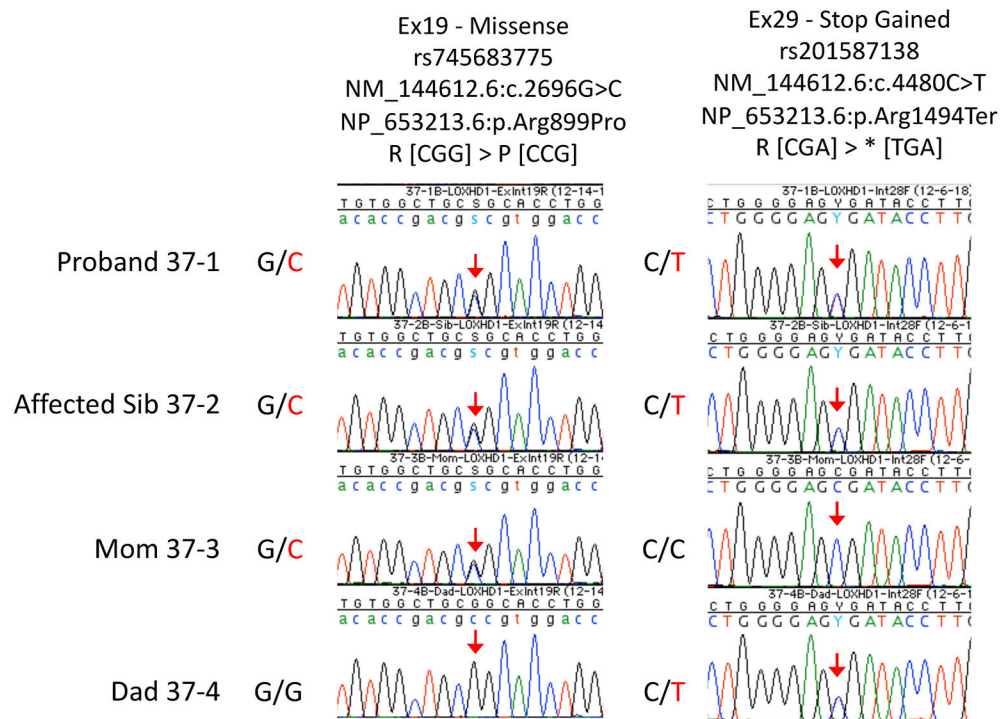


Fig. 2. Compound heterozygous *LOXHD1* mutations in proband (37-1) and affected sibling (37-2). In exon 19, a heterozygous missense mutation was identified at c.2696G > C in 37-1, 37-2, and 37-3. In exon 29, a heterozygous stop gained mutation was identified at c.4480C > T in 37-1, 37-2, 37-4. The red arrow shows the nucleotide where the mutation occurs within the chromatograms.